Studies on Autolysis Cells Induced by Phosphate Deficiency of the Cyanobacterium Synechocystis sp. PCC 6803

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Abstract

In the cultivation of microalgae for the production of useful compounds, cell disruption to extract the products of interest is a bottleneck and costly process. To establish a cost-effective method to recover these cellular compounds, I developed a method to induce cell lysis via phosphate deficiency in the cyanobacterium *Synechocystis* sp. PCC 6803. In this system, the promoter from the *phoA* gene for alkaline phosphatase regulates the expression of bacteriophage genes encoding the lytic enzymes holin and endolysin, and is induced under phosphate-deficient condition.

I observed that 90% of the transformant *lic* (*l*ysis *i*nducible *c*ells) were lysed after 24 h of incubation under phosphate-deficient conditions. Also, a considerable increase in proteins in the culture medium supernatant was observed. On the contrary, almost no cells of the all strains were lysed during the 3 day culture period when cultured in phosphate-sufficient culture, suggesting that the expression of the lysis genes was completely suppressed in the *lic* cells, and their viability was similar to those of the control cells.

I also attempted to lyse the cells at a higher cell concentration to demonstrate the practical usefulness of the lysis-inducible strain for the efficient recovery of cell products. The key point for the successful lysis in concentrated culture was pre-induction of the cell lysis by phosphate-deficiency before cell concentration, as previous study and my results suggest that lysis induction requires a certain level of light irradiation. Thus, I optimized cell culture

conditions to induce cell lysis and observed over 90% cell lysis after 16 h of incubation after culture concentration.

Interestingly, I found that the cell size and density of the *lic* cells were larger than the control cells even the cell lysis was not induced. And the precipitation efficiency of the *lic* strain was higher than controls. The mechanism of the cell enlargement is required further research. However, the characteristic of the fast precipitation is desirable traits for lesser energy operation of the cell collection.

In this lysis system, transition of growth phase to lysis phase occurs naturally with phosphate consumption by the cells, requiring no additional steps for inducing lysis gene expression. This system allows to reuse culture medium for another culture to save water resource, and may contribute to decrease cell disruption and extraction costs in the algal biotechnology industry.